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CUGC for Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome

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1. Disease characteristics

1.1 Name of the Disease (Synonyms):

Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome

1.2 OMIM# of the Disease:

238970

1.3 Name of the Analysed Genes or DNA/Chromosome Segments:

SLC25A15 or ORNT1 at 13q14.11

1.4 OMIM# of the Gene(s):

603861

1.5 Mutational Spectrum:

From 1999 to date, 39 mutations have been identified and associated to HHH syndrome.[1-5] All mutations are located in the coding region and consist of: 24 missense mutations, 5 small insertions, 3 small deletions, 4 nonsense mutations, 1 gross deletion, 1 micro-rearrangement, 1 intronic rearrangement.

1.6 Analytical Methods:

Sanger sequencing of coding regions and flanking intronic sequences of the *SLC25A15* gene is the mainstay analytical method (NCBI reference sequence: NG_012248.1).

1.7 Analytical Validation

There are several steps in the analytical validation process.

- Sequencing of both DNA strands (forward and reverse) is performed. Although most of variants have been identified by using this approach, intronic variants, large deletions, and duplications may require a different methodology.
- It is recommended to check the identified heterozygous or homozygous variants also in patients' parents. Proof of segregation further supports the diagnosis.
- Pathogenicity of variants can be investigated by using an *in vitro* yeast cell model[6] and by reconstitution of the bacterial-expressed recombinant mutant transporters into artificial phospholipids vesicles.[7]

1.8 Estimated Frequency of the Disease

(Incidence at birth ("birth prevalence") or population prevalence. If known to be variable between ethnic groups, please report):

Up to date, more than 114 affected patients have been reported.[2-4] In a recent review of 456 Urea Cycle Disorders (UCDs) patients prospectively followed between 2011 and 2015 by the E-IMD patient registry, 11 HHH syndrome patients were reported; thus, this disorder accounted for 2.4 % of all UCDs subjects.[8] Although the HHH syndrome can be considered a very rare disease with a panethnic distribution, it has been more frequently reported in three countries: 26 patients (22%) were Canadian, as a result of a founder mutation in Quebec[1], 18 patients (15%) were Italian and 14 patients (12%) were Japanese. In these three countries the HHH syndrome should not be considered a very rare disease since they account for about the 50% of all reported patients.

1.9 Diagnostic Setting:

	Yes.	No.
A. (Differential) diagnostics	☒	☐
B. Predictive Testing	☒	☐

C. Risk assessment in Relatives ☒ ☐
D. Prenatal ☒ ☐

Comment: HHH syndrome is a rare recessive UCD[9] caused by mutations in the *SLC25A15* gene [1-2,5], which encodes for the mitochondrial ornithine carrier ORC1.[1,6] Disease onset usually ranges from early infancy to childhood and, in rarer cases, adulthood. Patients typically present a variable clinical spectrum, ranging from a mild form with aversion for protein rich foods, developmental delay/intellectual disability, myoclonic seizures, ataxia and pyramidal dysfunction, to a more severe acute form with intermittent episodes of vomiting, confusion or coma, acute liver failure or hepatitis-like attacks.[3] The chronic course of HHH syndrome is characterized by progressive encephalopathy with mental regression, and early signs of motor neuron dysfunction (spastic paraparesis).[2,3] Multiple supratentorial stroke-like lesions have also been reported, while ophthalmological signs are rare, with few patients showing severe retinal degeneration.[3,10] The late onset cases present with hyperammonemia accompanied by lethargy and/ or coma and signs of severe liver dysfunction and /or life-threatening episodes of acute liver failure.[3]
Asides from the severe neonatal form, there is no evidence of a direct correlation between age of onset, which is variable, and disease severity. No clear phenotype–genotype correlations could be found. Early diagnosis in infancy or childhood may improve the clinical outcome. The diagnosis relies on clinical signs and the peculiar metabolic triad of hyperammonemia, hyperornithinemia, and urinary excretion of homocitrulline. Besides this biochemical signature, patients may also present with increased plasma glutamine levels and intermittent elevation of urinary orotic acid.[3] Some subjects also demonstrate low plasma creatine, as increased ornithine levels in this disorder inhibit arginine:glycine amidinotransferase, the first step of creatine synthesis.[10] Furthermore, secondary creatine deficiency may be due to low cellular arginine availability.[11] HHH syndrome enters in the differential diagnosis with other UCDs.[9]
Parents and relatives of HHH syndrome affected probands should be checked for the presence of the identified mutation(s). If a family has an affected child and wishes to have more children, prenatal diagnosis should be discussed in detail during genetic counselling.

2. Test characteristics

		genotype or disease: present	genotype or disease: absent
test	pos.	A	B
	neg.	C	D

A: true positives C: false negatives
B: false positives D: true negatives

sensitivity: A/(A+C)
specificity: D/(D+B)
pos. predict. value: A/(A+B)
neg. predict. value: D/(C+D)

2.1 Analytical Sensitivity
(proportion of positive tests if the genotype is present)

100%, if the genotype is also associated to at least one out of the three metabolic hallmarks, hyperammonemia, hyperornithinemia and homocitrullinuria.

2.2 Analytical Specificity
(proportion of negative tests if the genotype is not present)

100%, if the genotype is also associated to the absence of hyperammonemia, hyperornithinemia and homocitrullinuria.

2.3 Clinical Sensitivity
(proportion of positive tests if the disease is present)
The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

When the diagnosis has been properly established based on clinical and genetic investigations, family history and biochemical results, very few negative tests are expected. When genetic testing is negative in a patient presenting only one or two out of the three metabolic hallmarks, hyperammonemia, hyperornithinemia and homocitrullinuria, a differential diagnoses should be considered. Notably, some patients may show an incomplete biochemical phenotype and HHH syndrome presents with a lower degree of hyperammonemia compared to other UCDs.[3,9] These cases can likely result in a lower sensitivity.

2.4 Clinical Specificity

(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Close to 100%. A precise quantification is difficult, since molecular testing of *SLC25A15* gene is not performed on a routine basis in asymptomatic individuals.

2.5 Positive clinical predictive value

(life time risk to develop the disease if the test is positive)

The positive clinical predictive value is 100%. Although the onset of symptoms is in most cases in infancy and childhood, the diagnosis is often delayed, with at least one fourth of cases identified in adulthood.[3] Furthermore, a few patients presented the first disease manifestation as adults. As already described, genetic analysis does not have a prognostic value, since even in the same family and with the same mutation, the phenotype can be quite variable.[3]

2.6 Negative clinical predictive value

(Probability not to develop the disease if the test is negative).

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

The negative clinical predictive value is nearly 100%.

Index case in that family had not been tested:

Genetic testing for a clinically unaffected individual is not indicated in this situation. It would only be undertaken if a variant in the *SLC25A15* gene has been identified in the proband.

3. Clinical Utility

3.1 (Differential) diagnostics: The tested person is clinically affected

(To be answered if in 1.9 "A" was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No. ☐ (continue with 3.1.4)

Yes, ☒

- clinically. ☒
- imaging. ☐
- endoscopy. ☐
- biochemistry. ☒
- electrophysiology. ☐
- other (please describe):

3.1.2 Describe the burden of alternative diagnostic methods to the patient

The blood and urine samplings for the serum ammonia and ornithine and urine homocitrulline (and orotic acid) measurements is a minor burden to the patient. The blood sampling is also used for the genetic analysis which helps to confirm the clinical diagnosis.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

In some countries, like United States and Canada, HHH syndrome is included in the disease panel of expanded newborn screening programs, in others, like Italy, the cost of these tests is largely carried by the National Ministry of Public Health. Cost of biochemical and of genetic testing vary in different countries; however, since the *SLC25A15* gene is small and thus accessible to Sanger sequencing, costs of genetic testing are low in comparison to a too late diagnosis during a metabolic crisis requiring intensive care treatment.

3.1.4 Will disease management be influenced by the result of a genetic test?

No. ☐

Yes. ☒

Therapy (please describe)	An appropriate management with protein restricted diet, citrulline, arginine and essential aminoacid supplements combined with ammonia scavengers allows almost normal life duration. Chronic therapy prevents hyperammonemia and liver disease but does not affect the spastic paraparesis. [3] Acute treatment is similar to other UCDs.[9] Protein intake must be stopped for 24 hours, and intravenous fluids in combination with first-line medications must be initiated. Arginine (and/ or citrulline) must be administered to replace the missing urea cycle intermediates and to allow protein synthesis. Ammonia scavengers, sodium benzoate and sodium phenylbutyrate, are used for bypassing the urea cycle. Benzoate captures glycine to generate hippurate; phenylacetate is conjugated with glutamine generating phenylacetylglutamine. Long-term treatment consists of a low protein diet supplemented with citrulline, arginine, or ornithine. Protein restriction may be combined with sodium benzoate.[3,9] If plasma creatine levels are low, creatine supplementation should be instituted.[10,11] Citrulline supplementation has been shown to allow better metabolic control and to avoid secondary creatine deficiency.[3,11]
Prognosis (please describe)	Prognosis is highly variable ranging from mild neurological involvement to a severely disabling disease. 7 patients out of the published 114 patients, with a known vital status, died. Successful pregnancies have been reported in HHH syndrome female patients.[3]
Management (please describe)	Since HHH syndrome can affect different systems, follow-up by a multidisciplinary team is crucial. In particular, as the evolution of spastic paraparesis is unaffected by pharmacological treatment, regular physical and occupational therapy as well as drugs able to reduce spasticity are recommended; periodical clinical and neurophysiological evaluation of the progression of the disease is advisable as in other inherited spastic parapaplegias.[12] The metabolic follow-up includes measurement of plasma ammonia and amino acids (and possibly creatine) and urinary homocitrulline and orotic acid.

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history

(To be answered if in 1.9 "B" was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is **positive** (please describe) Although an appropriate and chronic therapy (see 3.1.4) prevents hyperammonemia and liver disease, it does not prevent the spastic paraparesis progression. If positive, the test will greatly influence the choice of career and life planning.

If the test result is **negative** (please describe) If the mutation is identified in the index case and not in the unaffected proband, regular examinations are not necessary. Follow-up is recommended only if the disease-causing mutation could not be identified.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

That person at-risk should avoid a protein-enriched diet in order to prevent life-threatening hyperammonemic crisis.

3.3 Genetic risk assessment in family members of a diseased person

(To be answered if in 1.9 "C" was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?

Yes, a molecular diagnosis in an affected individual can resolve the genetic situation in that family. A positive test in a patient may lead, at adult age, to test the carriership of his/her partner.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

No, if a disease-causing mutation is identified in the index patient, family members should be tested in order to avoid a possible mutation's spreading in the adult age.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

If a disease-causing mutation is identified in the index patient, it may help to quickly identify another affected relative since very often affected patients show an aversion to protein-rich foods.

3.4 Prenatal diagnosis

(To be answered if in 1.9 "D" was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

Yes. Prenatal diagnosis should be performed by molecular analysis.

4. If applicable, further consequences of testing

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

Genetic testing for *SLC25A15* variants will provide a molecular diagnosis, allowing an appropriate management of the patient, reducing the risk of hyperammonemia and metabolic decompensation. It will also define the inheritance patterns and enable effective genetic counselling.

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Conflict of Interest

The authors declare no conflict of interest.

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ABSTRACT:

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Review of the analytical and clinical validity as well as of the clinical utility of DNA-based testing for mutations in the *SLC25A15* gene(s) in ☒ diagnostic,
☒predictive and
☒prenatal settings and for
☒risk assessment in relatives.